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ANTI ARTHRITIC ACTIVITY OF TINOSPORA CORDIFOLIA LEAVES BY DENATURATION STUDIES

R.Ramya[1], R.Maheswari[2,*]
[1]Department of Biochemistry, K.M.G College of Arts & Science, Gudiyattam, Vellore District, Tamilnadu, India
[2*]Head, Department of Biochemistry, K.M.G College of Arts & Science, Gudiyattam, Vellore District, Tamilnadu, India.
Email: maheswarikmg2013@gmail.com

ABSTRACT
The study was designed to evaluate the phytochemical screening and anti-arthritic activity of various bark extract of Tinospora cordifolia. The main objective of our study is to evaluate in vitro anti arthritic activity of various bark extracts of Tinospora cordifolia by using BSA denaturation method. Our study displayed that different bark extracts of Tinospora cordifolia (MLE, ELE and CFE) were able to inhibit the protein denaturation and can be used as an anti arthritic agent. The percentage of protein denaturation by different bark extracts of Tinospora cordifolia (MLE, ELE and CFE) were found to be 23%, 36%, 43% (MLE), 19%, 28%, 37% (ELE) and 43%, 58%, 66% (CFE) at concentration 100µg, 250µg and 500µg etc. From the present studied it had been concluded that CFE, and MLE were exhibiting the potential capability to inhibit the denaturation of protein when compared with standard drug diclofenac sodium. The results indicate that the extract contains antioxidants and phytochemicals. Proximate analysis of Tinospora cordifolia showed wide variations but was in compliance to standard monographs. Qualitative analysis of Tinospora cordifolia extracts of stem part showed the presence of alkaloids, carbohydrates, glycosides, phytosterols, tannins, and saponin has found in watery extract.

Key words: *Tinospora cordifolia, Bark, Methanol, Ethanol, Chloroform Extract, Antiarthritic, Diclofenac Sodium, BSA.*

1. INTRODUCTION

Giloy (*Tinospora cordifolia* family Menispemaceae) is an indigenous, common shrub found in the Himalayas, tropical regions of India and particularly abundant in the dense forests of Chhattisgarh. *Tinospora cordiofolia* is a creeper with grayish stem and tubercles (small warts) on the surface. It is antipyretic, diuretic and anti-inflammatory. It constitute of several compound preparation. It is used in fever, urinary disorders, dyspepsia, secondary syphilis, rheumatism, constipation, tuberculosis, leprosy and general debility. It is also used in treatment of rheumatism and jaundice. It is a blood purifier and may be useful in AIDS and other immune disorders also. It is also being proposed for cancer patient before and after chemotherapy. The use of *Tinospora cordifolia* in the treatment of a variety of diseases in well documented. The present study included quantitative and qualitative evaluation of aerial part of *Tinospora codiofolia*. The quantitative evaluation parameter like moisture content, total ash value, acid insoluble ash, water soluble ash value, alcohol and aqueous extractive values were found to be 3%, 8%, 2%, 12%, 8% and 13% respectively. In qualitative evaluation, petroleum ether extract showed the presence of alkaloids, glycosides, carbohydrates, tannins, sterols, proteins and amino acids. The aqueous extract also showed

the presence of alkaloids, glycosides, carbohydrates, tannins, proteins, and amino acids. *Tinospora cordifolia* growing with Neem (*Azadirachta indica*) is called as NEEM GILOY has chemical composition as similar as neem as well as giloy and show better therapeutic properties (Chaudhari S, 2013). The bark is creamy white to grey in colour and deeply left spirally (Khosa R.L, 1971). However, cortex of root is divided in to outer thick walled and inner parenchymatous zone (Aiyer K.N, 1963). Fruits develop during winter (Nadkarni K.M, 1976). The stem of this plant is generally used to cure diabetes by regulating level of blood glucose (Sangeetha M.K, 2011). The anti-diabetic properties exhibited by this plant species are attributed due to the presence of alkaloids (Magnoflorine, Palmetine, Jatrorrhizine) (Patel M.B, 2011), tannins, cardiac glycosides, flavonoids, saponins, steroids etc., (Zinjarde S.S, 2011). *T. cordifolia* is well known for its immunomodulatory response. This property has been well documented by scientists (Tripathi YB, 1997; Bishayi B, 2002; Subramanian M, 2002).

2. MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Tinospora cordifolia bark extract was procured as a gift from the Medical Shop, all other chemicals were purchased from Sigma-Aldrich, Chemicals Pvt. Ltd, India. All other chemicals used were of good quality and analytical grade.

PREPARATION OF EXTRACT

Weigh 20 g of *Tinospora cordifolia* stem powder paste into a 250 ml round-bottomed flask. Add 50 ml of methanol and 60 ml of dichloromethane. Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separatory funnel. Wash this mixture containing bioactive compounds with three portions of 150 ml each with sodium chloride solution. Dry the organic layer over anhydrous magnesium sulfate. Filter and evaporate most of the solvent in vacuum without heating. Same procedure is followed for the extraction of MLE and CFE extracts.

PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary Phytochemical Screening has to be carried out for the identification of reducing sugars, pentoses, disaccharides, polysaccharides, proteins and amino acids phytosterols, polyphenols and carotenoids etc.

METHOD

Test solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05ml of test solution of various concentrations.

Test control solution (0.5ml) consist of 0.45ml of bovine serum albumin (5% w/v aqueous solution) and 0 .05ml of distilled water.

Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution.

Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05ml of Diclofenac sodium of various concentrations.

Various concentrations (100, 250, 500 µg/ml) of test extracts and standard drug diclofenac sodium (100, 250, 500 µg/ml) were taken respectively. All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37°C for 20 minutes and the temperatures were increased to keep the samples at 57°C for 3 minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 416 nm. The control represents 100% protein denaturation. The results were compared with Diclofenac sodium. All determinations were done in triplicate.

The percentage inhibition of protein denaturation can be calculated as:

Percentage Inhibition

$$= 100 - \frac{(\text{O. D of test solution} - \text{O. D of product control})}{\text{O. D of test control}} \times 100$$

Pharmacognostical evaluation

The stem part was subjected to proximate analysis. Quantitative standards like Moisture content, Total Ash value, Acid insoluble extractive values for aerial determined (Table 3, Table 4, Table 5).

a) Moisture content

The moisture content of a drug should be determined. Moisture content of the aerial part determined by using Infrared moisture balance Model – (Bell India Pvt. Ltd.) (Table 3).

b) Ash value

1) Total ash value

When vegetable drugs are incinerated, they leave an organic ash in some plants. The total ash usually contains carbonates, phosphate, silicate, and silica (Tabel 3)

Calculation

$$\textit{Ash \%} = (B - C) \times \frac{100}{A}$$

Where, A is weight of sample in gram
B is weight of dish + content after drying (g)
C is weight of empty dish (g)

2) Acid insoluble ash

Total ash treated with dilute hydrochloric acid reacts with minerals to form soluble salts and the insoluble ash consists mainly of silica, as acid insoluble ash (Table 3).

c) Extractive values

The determination of water and alcohol soluble extractive value was used as means of evaluating the quality and purity of the constituents. Extraction of the drug can be maceration with cold water or by continuous extraction procession a Soxhlet extractor (Tale 3, Table 4).

1) Alcohol soluble extractive values

2) Water soluble extractive values

Calculation

$$\frac{\textbf{The percentage of water soluble extractive values}}{\textbf{Alcohol soluble extractive values}} = \frac{\mathbf{B - A \times 4 \times 100}}{W}$$

Where, A= Empty weight of the dish (g)
B= Weight of dish + residue (g)
W= Weight of plant material taken (g)

Preparation of extraction

The stem part powder was subjected to systemic phytochemical screening by Extracting them with three solvents viz- petroleum ether and water. Then testing for the presence of chemical constituents.

Solvent extraction

The method is based on the extraction of active constituents present in the drug, using three solvents ranging from non-polar to polar. The solvents used petroleum ether & water. The extraction was done using soxhlet apparatus.

Procedure of Solvent Extraction

Extraction by soxhlet method

200 g of powdered bark extract was subjected drug was subjected to soxhlet extraction with two solvents, viz- petroleum ether and water for 6 hrs. All the extracts were concentrated by using rotary vacuum evaporator at low temperature. They were then weighed and percentage of different extractive values was calculated with respect to air-dried substance.

3. DISCUSSIONS

PHYTOCHEMICAL SCREENING

Preliminary Phytochemical screening of various extracts of papaya had shown the presence of various bioactive compounds such as carbohydrates (MLE, ELE and CFE), amino acids and peptides (MLE, ELE and CFE), phytosterols (CFE), carotenoids (MLE, ELE and CFE) and polyphenols (CFE) (Table 6).

BIOLOGICAL SCREENING

The in vitro anti arthritic results are using Bovine serum albumin denaturation method is given in Table 7 & Graph 1, Denaturation of protein is an important cause of rheumatoid arthritis which is well documented. Production of auto antigen in certain arthritis may be due to the denaturation of protein. The inhibition of denaturation of protein may one of possible target for the treatment of arthritis. Our study displayed that different extracts of *Tinospora cordifolia* (MLE, ELE and CFE) were able to inhibit the protein denaturation and can be used as an anti arthritic agent. The percentage of protein denaturation by different extracts of *Tinospora cordifolia* (MLE, ELE and CFE) were found to be 23%, 36%, 43% (MLE), 19%, 28%, 37% (ELE) and 43%, 58%, 66% (CFE) at concentration 100 µg, 250 µg and 500µg etc.

PHRMACOGNOSTICAL EVALUATION

The aerial part are subjected to quantitative standard (proximate analysis) like moisture content, total Ash value ,acid insoluble ash, extractive value and water soluble extractive values for sample were determined.

a) Moisture Content

Results of estimation of moisture content are tabulated in Table 3.

Analysis of the result is indicates that, in *Tinospora cordifolia* moisture content was maximum (3%)

b) Ash values

The total ash, acid insoluble ash and water-soluble ash values were determined for air dried samples using the procedure described in anonymous, quality methods for medicinal plant materials, WHO, Geneva. From the results, both the plant parts shows slight difference were tabulated in Table 3.

Proximate analysis of *Tinospora cordifolia* showed wide variations but was in compliance to standard monographs. Qualitative analysis of *Tinospora cordifolia* extracts of stem part showed the presence of alkaloids, carbohydrates, glycosides, phytosterols, tannins, and saponin has found in watery extract.

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4. CONCLUSION

The results obtained from the *in-vitro* studies performed by BSA denaturation method displayed that the various extracts of *Tinospora cordifolia* (MLE, ELE and CFE) possessed a very good anti arthritic activity. From the study it had been concluded that CFE, and MLE were exhibiting the potential capability to inhibit the denaturation of protein when compared with standard drug diclofenac sodium (Table 7 and Graph 1).

Proximate analysis of *Tinospora cordifolia* showed wide variations but was in compliance to standard monographs. Qualitative analysis of *Tinospora cordifolia* extracts of stem part showed the presence of alkaloids, carbohydrates, glycosides, phytosterols, tannins, and saponin has found in watery extract (Table 6).

5. RESULTS

Sample Identify	Moisture content %	Standard Value	Total Ash %	Standard Value	Acid Insoluble Ash %	Standard Value	H_2O Soluble Ash %	Standard Value
Tinospora cordifolia	3	NMT 6%	8	NMT 11%	2	NMT 4%	13	NMT 21%

Table 3: Data showing values of moisture content, total ash acid insoluble ash, and Water soluble ash in *Tinospora cordifolia*.

Sample Identify	% of Alcohol Soluble Extractive	Standard Value	% of Water Soluble Extractive	Standard Value
Tinospora cordifolia	8	NMT 2%	13	NMT 10%

Table 4: Data showing % Alcohol soluble extractive values and % water soluble extractive values in *Tinospora cordifolia* stem part.

Plant parts	Solvents	Colour	Nature of the extracts	Solubility
Stem Part	Petroleum ether	Dark brown	Sticky	Chlorofrom, DMSO
	Water	Black brown	Sticky	Water, DMSO

Table 5: Data showing successive extractive values and nature of extracts of the *Tinospora cordifolia* stem part.

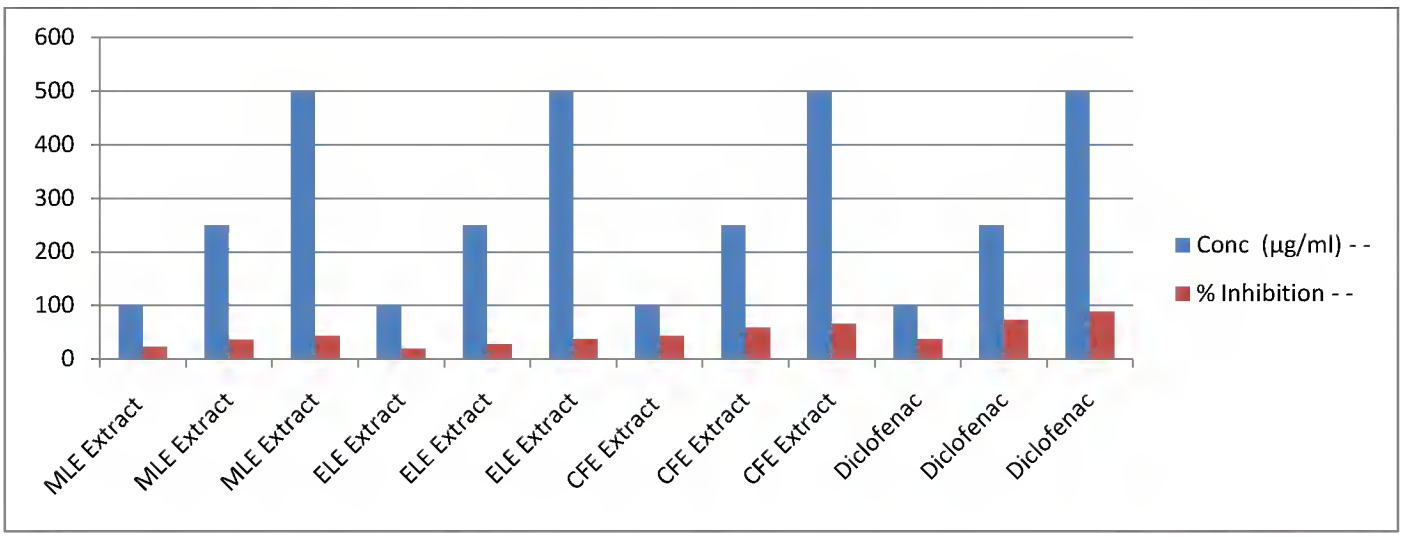
Chemical Constituent	Test's	Petroleum Ether	Aqueous
Alkaloids	Mayers test	+	+
	Dragendroff's test	+	+
	Wagners test	+	+
	Hagers test	+	+
Carbohydrates	Mollisch's test	+	+
	Benedicts test	+	+
Glycosides	Modified Borntragers	+	+
	Legal test	+	+
	Killer killiani test	+	+
Saponins	Foam test	-	+
	Froth test	-	+
Phytosterols	Salkowaski test	+	+
	Libbermann burchard test	+	+
Resins	Acetone water test	-	-
Phenols	Ferric chloride test	-	-
Tannins	Gelatin test	+	+
Flavanoids	Alkaline reagent	-	-

	Lead acetate test	-	-
	Shinoda test	-	-
Protiens	Ninhydrin test	-	-
	Xanyhoproteic test	-	-
	Biurete test	-	-

Table 6. Qualitative chemical tests of the extract of *Tinospora cordifolia.* [+Present, -Absent]

Drug	**Concentration (µg/ml)**	**% Inhibition**
Test Control	-	-
Product Control	-	-
MLE Extract (Test)	100	23.5±3.5
	250	36.6±4.3
	500	43.6±3.2
ELE Extract (Test)	100	19.7±2.5
	250	28.6±2.6
	500	37.4±3.3
CFEE Extract (Test)	100	43.7±4.2
	250	58.8±5.3
	500	66.9±4.4
Diclofenac Sodium (Standard Drug)	100	37.4±2.6
	250	73.5±3.6
	500	88.8±3.4

Table 7. Result of *In-vitro* anti-arthritic activity of drugs in BSA denaturation method
Values are in Mean ± SD, n=3.



Graph 1. result of *In-vitro* anti-arthritic activity of drugs in BSA denaturation method.

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